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Multiple dose protocol for the administration of GnRH-antagonists in IVF: the "Lübeck-protocol"

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SUMMARY

Due to their different pharmacological mode of action GnRH-antagonists are able to suppress serum-concentrations of LH within hours. Instead of "down-regulation" and "desensitization" a classic competitive blockage of the GnRH-receptors on the cell-membrane of the gonadotrophic cells seems to take place. The "flare up", typical for agonistic GnRH-analogues is completely avoided. While the first generation of GnRH-antagonists caused important problems due to allergic reactions, which inhibited their clinical introduction, Cetrorelix and Ganirelix as representatives of the youngest generation of these compounds seem to avoid these disturbances completely. Cetrorelix was introduced first in our IVF-program to scrutinize the possibility of avoiding premature LH-surges. All patients were treated with human menopausal gonadotrophins (HMG), starting on day 2. From day 7 until induction of ovulation by human chorionic gonadotrophins (HCG) Cetrorelix is administered s.c. in a daily fashion. Starting with a dosage of 3mg Cetrorelix/die no premature LH-surges could be observed. 1mg/die, 0,5mg/die and 0,25mg/die administered according to the described "Lübeck-protocol" also proved to avoid any premature LH-surges. The mean courses of FSH and LH in the different dosage groups were quite similar with a profound suppression of LH. Estradiol concentrations reflected a satisfactory follicular development. The fertilization-rate after IVF in cases of tubal infertility or ICSI in cases of male subfertility were within the range to be expected

after normal oocyte maturation. Avoiding any flare-up effect treatment cycles could be shortened significantly in comparison to an agonistic long-protocol-stimulation. 0,1mg Cetrirelix/die instead was not able to prevent premauture LH-surges. 0,25mg Cetrorelix/die is now used in the frame of a multicentric Phase III-study. Ganirelix is used at present time according to the described stimulation protocol within a phase-II-dose finding study.

INTRODUCTION

The disclosure of the neuroendocrine control of the menstrual cycle in adult women may be one of the most important challanges of the last three decades within gynecology with an outstanding clinical impact on treatment of hormonal disorders in gynecology and pediatrics, infertility treatment and oncology. The hypothalamus is the superordinate organ releasing the gonadotropin-releasing hormone (GnRH) in a pulsatile manner. GnRH is a peptide composed of 10 amino acids, which was first isolated and characterised in 1971 by Schally and Guillemin, who were awarded the Nobel prize for their pioneering work in 1977 [1, 2]. GnRH is secreted by the neural cells of the nucleus arcuatus in the mediobasel portion of the hypothalamus. The axons in these neurons are in intimate contact with the vessels of the hypothalamic-pituitary portal vein system. The pulsatile release of GnRH by the hypothalamic neurons causes the gonadotrophic cells of the pituitary gland, which make up about 10% of ist cell mass, to release the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) also in a pulsatile fashion. FSH and LH in turn control follicular maturation and gonadal sexual steroid biosynthesis.

GNRH-AGONISTS

After the amino-acid sequence of GnRH had been successfully isolated and analyzed it was possible by modification of the molecular structure of this decapeptide and by introduction of unnatural D-aminoacids, mainly at position 6 to obtain analog compounds with agonistic effects [3] These compounds have a 100-200 times higher binding affinity for the GnRH-receptors than the native molecule. The agonists, originally designed to enhance their affinity to the GnRH-receptors of the gonadotrophic cells as well as their duration of action, making them more resistant against enzymatic digestation, lead after a short period of stimulating the FSH and LH secretion, the so called "flare up", to a reduction of GnRH-receptors on the cell membrane of the gonadotrophic cells. As a result of the subsequent reduction in number of GnRH-receptors a paradoxical suppression of the pituitary gonadotrophin synthesis and liberation occurs. After a period of about 14 - 21 days of constant GnRH-agonists impact the pituitary gland becomes completely desensitized and refractory to a GnRH stimulus [4]. Decreased levels of LH and FSH result in the arrest of follicular development. This fall in gonadotrophins is followed by a fall of sex steroids to the castrate range. These phenomena represent the basis for the clinical use of GnRH-agonists.

GNRH-AGONISTS WITHIN CONTROLLED OVARIAN HYPERSTIMULATION (COH)

The occurence of premature LH-surges is a main reason for a relatively low efficacy of ovarian stimulation by HMG only in IVF-programs. In addition, these LH-surges have a negative impact on the quality of the oocytes and embryos and subsequently on the rate of pregnancy. By introducing the GnRH agonists into the stimulation protocols of Assisted-Reproduction-Technique-programs (ART-programs) an improved synchronization of follicular maturation and an important reduction of premature luteinization to lower than 2% could be achieved. The timing of the puncture has become fully calculable under this regime and can be managed in relation to clinical necessities as well as patients wishes. The long protocol synchronizes follicle maturation and makes it possible to select a larger number of follicles or oocytes for IVF than the other protocols [6]. The long protocol is most used at present for controlled ovarian hyperstimulation. However, it has the disadvantage of a long treatment period until desensitization occurs as well as of relatively high costs due to an increased requirement for HMG.

GNRH-ANTAGONISTS

In parallel with the development of GnRH-agonists other analogues were synthesized which also bind to the pituitary GnRH-receptors but are not functional in inducing the release of gonadotrophins. These compounds are far more complex than GnRH-agonists with modifications in the molecular structure not only at position 6 and 10, but also at position 1,2,3 and 8. In comparison to the GnRH-agonists the pharmacological mechanism by which GnRH-antagonists suppress the liberation of gonadotrophins is completely different. While the agonists act on chronic administration through down regulation of receptors and desensitization of the gonadotrophic cells, the antagonists bind competitivcly to the receptors and thereby prevent the endogenous GnRH from exerting its

GnRH-Antagonists: the "new generation"

	1	2	3	4	5	6	7	8	9	10
Cetrorelix	Ac-D-Nal(2)	D-Phe(4Cl)	D/PAL	SER	TYR	D/CIT	LEU	ARG	PRO	D-Ala-NH2
Ganirelix	Ac-D-Nal(2)	D-Phe(4Cl)	D/PAL	SER	TYR	D-hArg(Et2)	LEU	L-hArg	PRO	D-Ala-NH2

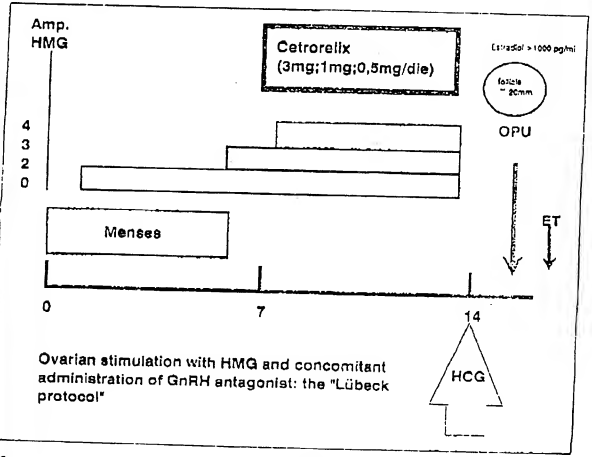
FIG. 1

stimulatory effects on the pituitary cells avoiding any „flare up effect". Within hours the secretion of gonadotrophines comes down. This mechanism of action is dependent on the equilibrium between endogenous GnRH and the applied antagonist. Due to this antagonists effect is highly dose depending in contrast to the agonists.

While in the first generation of GnRH-antagonists allergic side effects due to an induced histamine release hampered the clinical development of these compounds [14], modern GnRH antagonists like the Ganirelix (Oganon, Oss, Netherlands) or Cetrorelix (ASTA-Medica, Frankfurt/M, Germany) seem to have solved these problems and thus may become available medically in the near future, both of them having been used at our department [6]. (Fig. 1)

GNRH-ANTAGONISTS WITHIN CONTROLLED OVARIAN HYPERSTIMULATION (COH): THE "LÜBECK-PROTOCOL"

In 1991 Dittkoff et al. showed that a GnRH-antagonist applied for a short period is capable of suppressing the ovulation-inducing mid-cycle LH-peak [7]. They administered 50 µg of Nal-Glu per kg body weight and day for four days in the midcycle phase. The LH peak failed to occur,



Ovarian stimulation with HMG and concomitant administration of GnRH antagonist: the "Lübeck protocol"

FIG. 2

estradiol production came to halt and follicular growth was interrupted. After discontinuing the antagonists, gonadal function normalized within days. Apparently, antagonists neither deplete the FSH and LH stores of gonadotrophic cells nor inhibit gonadotropin synthesis.

Transferring these results into a protocol of controlled ovarian hyperstimulation with HMG to avoid the onset of premature luteinization the premature LH surge seems to be abolished as well by daily administration of the modern GnRH-antagonist Cetrorelix from day 7 onward until ovulation induction, what we call the „Lübeck"-protocol" (Fig.II), as by single or dual administration around day 9. In this protocol the antagonist is injected at the time when estradiol reaches 150-200 pg/ml and the follicle size is >14mm, which usually is the case on day 9 of the cycle [8]. Until now over 730 patients have been treated by these protocols and both have been proven to be safe and effective. The discussion about advantages and disadvantages of the two possible ways of administration is still going on, although we favorize the „Lübeck protocol" due to its major stability preserving all the advantages regarding therapys comfort of the „long" agonistic protocol we are accustomed to.

To elucidate the question of the dosage necessary for sufficient suppression of the pituitary gland at this critical monient of controlled ovarian hyperstimulation in two subsequent open phase II studies three dosages were administered in accordance to the „Lübeck protocol" and the hormone profiles obtained as well as the number of oocytes retrieved, the fertilization rates and the consumption of HMG were compared.

35 patients, all suffering fom tubal infertility and no other infertility factors being observed were treated as follows: Starting on cycle day 2 stimulation started with two ampoules of HMG daily. From cycle day 7 until induction of ovulation 12 patients were treated with 3mg Cetrorelix s.c./day. As no premature LH surge could be observed, 12 patients received 1mg Cetrorelix/day, and another 11 patients 0,5mg Cetrorelix/day. On day 5 the dose of HMG was adjusted to the individual ovarian response of the patient to the stimulation as assessed by estradiol values and measurement of follicles.This treatment was continued until induction of ovulation with 10.000 IU HCG i.m., given when the leading follicle reached a diameter of 18-20mm, measured by transvaginal ultrasound, and when estradiol values indicated a satisfactory follicular response.

No premature LH-surge was observed. All cycles could be evaluated. The mean courses in the three dosage groups of FSH and LH were quite similar with a profound suppression of LH and a less pronounced suppression of FSH, the latter observation probably due to the fact of the larger half-plasma life of the injected FSH. In the case of estradiol there was a distinctly higher increase in concentration in the group treated with 0,5mg Cetrorelix/day, reaching an average maximum of 2165pg/ml on cycle day 10, compared to 852 pg/ml in the 3-mg group and 1023 pg/ml in the 1-mg group. Although not being significant these differences seem to indicate a slightly more sensitive reaction to the stimulation with HMG in the group treated with the lowest dosage of antagonist compared to the other.

The fertilization rates of the recovered oocytes were 45,3% in the 3-mg group, 53,2% in the 1-mg group and 67,7% in the 0,5-mg group. In the 3-mg group 106 oocytes were recovered and 30 embryos were obtained, 36,7% of them being excellent according to morphological microscopic criteria. In the 1-mg group 94 oocytes were collected and 28 embryos obtained, 53,6% being excellent. In the 0,5-mg group 127 oocytes were recovered and 27 embryos were obtained, 37% of them being excellent (Tabl.I).

TAB. I

Ovarian stimulation with HMG and Cetrorelix (3mg;1mg;0,5mg)

Recovered oocytes, fertilization rate, No. and quality of embryos

	3mg	1mg	0,5mg
No. of oocytes	106	94	127
fertilization rate	45,3%	53,2%	67,7%
No. of embryos	30	28	27
Excellent embryos	36,7%	53,6%	37%

The average use of HMG ampoules was 30 in the 3-mg group, 27 in the 1-mg group and 26 in the 0,5-mg group. This differences are not significant, but have to be compared with the quite higher amount of ampoules used in an agonistic „long protocol" [9].

Subsequent dose finding studies using as well 0,5mg Cetrorelix/die as 0,25mg Cetrorelix/die and 0,1mg Cetrorelix/die proved the efficacy and safety of 0,25mg Cetrorelix/die in avoiding premature LH-surges, while under 0,1mg Cetrorelix/die premature LH-surges could be observed [10]. In these studies ICSI for treatment of male subfertility of the husband was allowed, leading to fertilization rates within the range to be expected after normal oocyte maturation.

Up to now 238 patients have been treated within Phase II studies, using Cetrorelix, 134 patients according to the single/dual-dose protocol and 104 patients according to the multiple dose protocol. 69 pregnancies could be achieved, meaning a pregnancy rate of 30%.

0,25mg Cetrorelix/die as the minimal effective daily multiple dosage is now used in the frame of a multicentric Phase III-study. Ganirelix is used at present time according to the described stimulation protocol withi a phase-II-dose finding study.

Serum concentrations of Cetrorelix showed to be dose-dependent concerning as well the concentration after first administration as maximal concentrations to be measured. Also concentrations of Cetrorelix in the follicular fluid showed to be dose-dependent.

Based on the mechanism of competitive binding, it is possible to modulate the degree of hormone suppression by the dose of antagonist to be administered. This preservation of the pituitary response could be clearly demonstrated [11]. This can open new paths in the treatment of patients at higher risk for developing an Ovarian Hyperstimulation Syndrome (OHSS), as it would allow the avoidance of in some cases deleterious effects of HCG administration. Ovulation induction should be possible by GnRH-agonists or native GnRH itself under antagonistic treatment. This could help to lower the incidence rate of early onset-OHSS.

CONCLUSIONS

It is still too early to speculate about the possible end of the agonist „era". GnRH agonists are valuable and safe pharmaceutical tools within controlled ovarian stimulation protocols. Safety and efficacy of the antagonists hae still to be proven in long-term studies. However, from what is known until now, the advantages of GnRH-antagonists are evident. After having been introduced into the market they probably will replace completely the agonists for this indication.

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